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(54) Title: ENZYMATIC DEGREASING OF SKINS AND HIDES

(57) Abstract

The present invention relates to a method of degreasing skins and hides during the processing of skins and hides into leather. More specifically the invention relates to a process for enzymatic degreasing of skins and hides which comprises treatment with a lipolytic enzyme at a pH below 5.

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ENZYMATIC DEGREASING OF SKINS AND HIDES

TECHNICAL FIELD

The present invention relates to a method of degreasing skins and hides during the processing of skins and hides into leather. More specifically the invention relates to a process for enzymatic degreasing of skins and hides comprising treatment with a lipolytic enzyme at a pH below 5.

BACKGROUND ART

Skins and hides contain regions of natural fat. However, excess fat needs to be reduced during the leather manufacturing process in order to achieve a satisfactory finish of the final leather product.

Degreasing of skins and hides is currently accomplished by use of organic solvents and surfactants.

Recently the use of lipolytic enzymes in order to improve degreasing of hides and skins has been suggested, thereby reducing or even avoiding the use of surfactants or as a substitute for organic solvents. When compared to traditional methods, enzymatic degreasing processes generally improve the quality of the final leather, reduce the use of chemicals and replace chemicals which have an adverse effect on the environment.

Lipolytic enzymes hydrolyse fats into mono- and diglycerides, free fatty acids and glycerol. At alkaline conditions the mono- and diglycerides produced by the lipase are considered having an emulsifying effect. At pH below 8 deposit of free fatty acids may appear.

Enzymatic degreasing processes are suggested performed at a pH in the range of 5-13. However, it is recommended that the degreasing process is carried out at a pH above 7, preferably the range of pH 8-10.

Known enzymatic degreasing processes therefore have taken into account the improved emulsifying effect arising from carrying out the degreasing process at alkaline conditions. However, enzymatic degreasing processes carried out at a pH below 5 have never been suggested.

SUMMARY OF THE INVENTION

The present invention provides a process for enzymatic degreasing of skins and hides, comprising treatment with a lipolytic enzyme at a pH below 5. The process of the invention improves degreasing of skins and hides.

The process of the present invention improves the chromium uptake and dispersal in hides/skins thereby reducing the amount of chromium in the waste water, and it also improves uptake of dye-stuff, fat liquors, etc.

By the process of the invention, the need for pH adjustment becomes considerably reduced, thereby saving chemicals and time, mainly because of the extensive buffer effect of skins and hides.

Accordingly, in its first aspect, the invention provides a process for enzymatic degreasing of skins and hides, which process comprises treatment of the skin or hide with a lipolytic enzyme at a pH below 5.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is further illustrated by reference to the accompanying drawing, in which:

Fig. 1 shows the relation between lipolytic activity on long chain substrates and the lipolytic activity on tributyrine substrate. The figure shows the absorbency at 406 nm (OD_{406}) determined at pH 3 and 25°C with three different lipolytic enzymes (■ a lipase derived from *Candida antarctica*, □ a lipase derived from *Candida cylindracea*, and ♦ a lipase derived from *Pseudomonas cepacia*) in the test for lipolytic activity according to Example 1 of this specification.

Fig. 2 shows the brightness determination carried out according to Example 2 of this specification. The brightness is expressed by as L*-value x 100 (between 5800 and 6040). The figure shows two determinations, which each represents an overall average from 6 skins processed. "y" represents the brightness determination of the skins processed according to the method of the invention (lipase present) and "n" represents the brightness determination of the skins processed with no lipase present. The higher the L*-value the brighter the colour of the skins;

Fig. 3 shows the brightness determination carried out according to Example 2 of this specification. The brightness is expressed by as L*-value x 100 (between 6300 and 6600). The figure shows two determinations, which each represents an overall average from 6 skins processed. "y" represents the brightness determination of the skins processed according to the method of the invention (lipase present) and "n" represents the brightness determination of the skins processed with no lipase present. The higher the L*-value the brighter the colour of the skins; and

Fig. 4 shows the colour determination carried out according to Example 2 of this specification. The colour is expressed by an a*-value x 100 (between 900 and 1500). The figure shows two determinations, which each represents an overall average from 6 skins processed. "y" represents the colour determination of the skins processed according to the method of the invention (lipase present) and "n" represents the colour determination of the skins processed with no lipase present. The higher the a*-value the more red the colour of the skins.

DETAILED DISCLOSURE OF THE INVENTION

The present invention provides a process for enzymatic degreasing of skins and hides, comprising treatment with a lipolytic enzyme at acidic conditions.

In the context of this invention the term lipolytic enzymes includes lipases and esterases.

Enzymatic Degreasing

The process of the present invention may be applied to any skin or hide conventionally used for leather manufacturing. In particular, the process of the invention may be applied to ovine skins, to porcine skins, to bovine hides, or to caprine skins.

Enzymatic degreasing according to the present invention may take place any time during the manufacture of leather, either as a separate step or as part of an existing leather processing step. However, the process preferably takes place during, or in between, process steps that are carried out at acidic conditions, in order to avoid unnecessary and time consuming pH adjustment.

The process of the present invention is carried out at pH below 5. The process is preferably carried out at a pH in the range of from about pH 2 to about pH 4.5. In its most preferred embodiment the process of the invention is carried out at a pH in the range of from about pH 3 to about pH 4.

In a preferred embodiment, the process of the invention for enzymatic degreasing takes place during one or more of the subsequent steps of bating, pickling and tanning.

In another preferred embodiment, the process of the invention for enzymatic degreasing takes place as a separate step, performed any period of time after pickling has been finished, but before tanning is initiated. At the end of the pickling step, pH of the reaction mixture is usually in the range of pH 1-3 (around pH 2). When the process of the invention takes place after the pickling step, pH is preferably adjusted to a pH in the range of pH 3-4.5.

In yet another preferred embodiment, the process of the invention for enzymatic degreasing takes place as a separate step, performed any period of time after tanning has been finished.

The process of the invention may be carried out at temperatures normally employed in leather manufacturing processes, i.e. in the range of from about 15 to about 65°C, or even up to about 75°C. Dependent i.a. on the hide or skin in question the temperature preferably is kept in the range of from about 20 to about 45°C (in particular when applied to ovine hides), or in the range of from about 25 to about 65°C (in particular when applied to bovine hides).

Enzymatic Treatment of Skins and Hides

In a process according to the present invention for enzymatic degreasing of skins and hides, the skin or hide is treated with a lipolytic enzyme in an aqueous reaction medium, in order to hydrolyse fats present in the skin or hide.

The treatment may take place in the presence or absence of surfactants. The surfactant preferably is an anionic, a non-ionic, or an amphoteric type surfactant, or a mixture thereof. Moreover, organic solvents may be present during the lipolytic treatment, but organic solvents are not needed in a process of the invention. Therefore, out of environmental concern, the reaction mixture should be kept free of organic solvents.

The reaction time greatly depends on the enzyme dosage and to a lesser degree on the temperature. For practical reasons a reaction time in the range of 30 minutes to 24 hours is contemplated. Preferably the reaction time is in the range of ½-16 hours, more preferred ½-4 hours, most preferred ½-2 hours.

When hydrolysis takes place, hydrolysis products are formed. These reaction products should be removed from hides and skins.

Hydrolysis products may be removed by separating hides and skins from the aqueous reaction medium. Preferably the hides and skins are subsequently washed repeatedly with water, preferably in the presence of conventional surface active agents.

The surface active agent used in an aqueous mixture for removal of the hydrolysis products may be any conventional surfactant. However, anionic, non-ionic and amphoteric type surfactants are preferred, either as separate surfactants or in mixture.

Lipolytic Enzymes

The lipolytic enzyme used according to the present invention may be any lipase and/or esterase having lipolytic activity on fats and long chain substrates at low pH. More particularly, the lipolytic enzyme is a lipase and/or an esterase having activity on fats and long chain substrates at a pH in the range of pH 1-5, more preferred the range of pH 2-5, most preferred pH 3-4.5.

In the context of this invention, a long-chain substrate is a mono-, di- and/or triacylglycerols having a chain of 12 or more carbon atoms. The substrate may be saturated or unsaturated.

The lipolytic enzyme may be a specific reacting lipase and/or esterase or it may be an unspecific reacting lipase and/or esterase. The lipolytic enzyme is preferably an unspecific reacting lipase and/or esterase, reacting at triacylglycerols, phospholipids and/or cholesterolesters as well.

In a particular embodiment, the lipolytic enzyme used may be one or more of the lipases and/or esterases derived from *Aspergillus*, in particular *Aspergillus niger* and *Aspergillus flavus*, *Achromobacter*, in particular *Achromobacter iophagus*, *Bacillus*, in particular *Bacillus pumilus* and *Bacillus strearothermophilus*, *Candida*, in particular *Candida cylindracea* (*Candida rugosa*), *Candida paralypolitica*, and *Candida antarctica*, *Chromobacter*, in particular *Chromobacter viscosum*, *Fusarium*, in particular *Fusarium oxysporum*, *Humicola*, in particular *Humicola brevispora*, *Humicola brevis* var. thermoidea, and *Humicola insolens*, *Hyphozyma*, *Pseudomonas*, in particular *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Pseudomonas fragi*, *Pseudomonas mephitica*, *Pseudomonas alcaligenes*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas putida*, *Pseudomonas mendocina*, or *Pseudomonas stutzeri*, *Rhizomucor*, in particular *Rhizomucor miehei*, *Rhizopus*, in particular *Rhizopus nodosus*, and/or *Thermomyces*, in particular *Thermomyces lanuginosus* (formerly *Humicola lanuginosa*).

In a most preferred embodiment, the lipolytic enzyme used according to the invention is derived from *Candida cylindracea*, in particular *Candida cylindracea* Lipase A (obtained as described in Example 10 of WO 88/02775), *Candida antarctica*, *Pseudomonas cepacia*, and/or *Hyphozyma*.

The lipolytic enzyme of the invention may be applied in concentrations conventionally employed in degreasing processes. It is at present contemplated that the lipolytic enzyme may be added in an amount of from 2.5 to 500 LU per g of hide or skin, preferably of from 5 to 250 LU per g of hide or skin.

Lipolytic Activity

The lipolytic activity may be determined using tributyrine as substrate. This method is based on the hydrolysis of tributyrine by the enzyme, and the alkali consumption is registered as a function of time.

One Lipase Unit (LU) is defined as the amount of enzyme which, under standard conditions (i.e. at 30.0°C; pH 7.0; with Gum Arabic as emulsifier and tributyrine as substrate) liberates 1 μmol tithable butyric acid per minute.

A folder AF 300/1 describing this analytical method in more detail is available upon request to Novo Nordisk A/S, Denmark, which folder is hereby included by reference.

EXAMPLES

The invention is further illustrated with reference to the following examples, which are not intended to be in any way limiting to the scope of the invention as claimed.

Example 1

Determination of Lipolytic Activity at Low pH

In this example the lipolytic activity at low pH of three lipases is determined. The lipolytic activity was measured spectrophotometrically using a pNP-assay, in which the substrate is a mixture of p-nitro-phenyl-palmitate and trioleine.

The bottom of a quartz cuvette is coated with substrate by addition of 0.1 ml of a mixture of 5 mM p-nitro-phenyl-palmitate and 25 mM trioleine in hexane, followed by evaporation of the hexane.

Three solutions of lipolytic enzymes having an activity in the range of 0-5 LU/ml (cf. Fig. 1) in Citrate Buffer pH 3, Merck (25°C) were prepared with a lipase from *Candida cylindracea* (= *Candida rugosa*), obtained from Nippon Oil & Fats Co. Ltd., Japan, a lipase from *Candida antarctica*, Lipase A obtained according to WO 88/02775, and a lipase/esterase from *Pseudomonas cepacia*, obtained according to WO 93/10224, respectively.

1.5 ml of enzyme solution are added to the substrate, and the reaction mixture is incubated for 1 hour at 25°C. After incubation, 620 µl 1M TRIS buffer pH 10.5 (Aldrich) are added, and after exactly 10 seconds, the absorbency at 406 nm (OD_{406}) is determined (Hewlett Packard HP8452A Diode Array spectrophotometer).

OD_{406} is an expression of the lipolytic activity.

The results are presented in Fig. 1. When carried out on a blank, an OD_{406} of less than 0.025 was obtained, indicating a very limited auto-hydrolysis of the substrate.

It appears from the figure that all lipolytic enzymes investigated show a significantly hydrolytic activity (i.e. an OD_{406} of more than 0.2) at pH 3 on long chain substrate.

Example 2

Enzymatic Degreasing of Hides and Pelts

This example demonstrates the process of the invention as applied to pickled New Zealand lamb pelts. The lamb pelts are subjected to lipolytic treatment with a lipolytic enzyme derived from *Candida cylindracea* (*Candida rugosa*), obtained from Meito Sangyo, Japan.

When sheep and lamb skins are pickled, the fat cells will after a certain period of time, often around 14 days, be destroyed and the fat is more easily accessible for surfactants and enzymes.

The example is carried out as two trials running in parallel in two Dose tanning pilot drums: One drum with 6 pelts being treated with tenside only, and the other drum with 6 pelts being treated with both tensides and lipase. The 6 pelts in each drum were treated according to Recipe 1A and 1B, below.

After chrome tannage, the pelts were dried and shaved, thereby removing residual flesh and fat from the flesh side. Their colour was determined using a Minolta Chroma Meter (CR200), showing the lipase treated pelts to be more dark blue than the pelts not lipase treated, indicating a better chrome uptake of the lipase treated pelts (cf. Fig 2).

Samples were cut from the skins and analyzed for grease content in a Soxtech™ equipment (available from Tecator, Sweden), showing very significant

differences in grease content and composition. The results are presented in Table 1, below. The table shows the amount of grease present and the acid value after the Soxtech™ extraction for 24 hours with petroleum ether and titration with 0.01N KOH. Non-lipase treated leather (chrome tanned) contained 17% of grease having an acid value of 5.9, in contrast to the lipase treated leather which contained 7% of grease with an acid value of 111.

The pelt was subsequently made into dyed crust leather, cf. Recipe 1B. The colour of the crust leather was determined using Minolta Chroma Meter (CR200), which showed that the lipase treated pelts were darker compared to the non-lipase treated pelts (lower L* value), cf. Fig.3, and also more brown (higher a* value), cf. Fig.4. This indicates that the lipase treated pelts have taken up more dyestuff than the non-lipase treated pelts.

Short time after starting the drums, the skins deposited a substantial amount of grease on the inner surfaces of the drums. After addition of lipase (i.e. 2 hours before addition of the tenside), the grease started to disappear. When the combined lipase and tenside treatment was almost finished, the float was milky white, the pelts were spongy, a bit rough on the grain and more greasy on the flesh side.

During chrome tannage at the raised temperature, considerable amount of grease was released into the float and a "lamb roast" smell appeared.

The leathers were rehydrated just before shaving. Interestingly, a pronounced hydrophobicity of the lipase treated skins was observed.

Recipe 1A

Process	Compound	%	Temperature	Time	Remarks
Depickling	water	175	30		
	salt	8.0		15 min	
	HCOONa	0.85		30 min	pH 3.2 - 3.3
Degreasing	lipase	0.12 or 0		120 min	
	+water	150	30		
	Tergolix™ CA	0.5		15 min	
Drainage					
Washing	water	150	30		
	Tergolix™ CA	0.5		20 min	
Drainage					
Washing	water	300	30		
	Tergolix™ CA	1.0		30 min	
Drainage					
Washing	water	150	30		
	Tergolix™ CA	0.5		15 min	
Drainage					
Chrome tannage	water	100	20		
	salt	6		10 min	
	formic acid (96%) diluted i float before add.	0.5		30 min	pH 2.5-3.0
	Chromosal™ B	3.5		30 min	
	Baychrome™ CP	2.4		over night	
Drainage					
Washing	water	200	25	15 min	
Drainage					

Skins are drained, dried and shaved, and the product is referred to as wet blue skins.

Recipe 1B

Process	Compound	%	Temperature	Time	Remarks
Retannage					
Washing	water	300	40		
	formic acid (96%) diluted	0.094		20 min	
Drainage					
Neutralizatio n	water	200	40		
	Na formiate	1.5			
	Tanigan™ PAK-N fl	1.5		60 min	pH 5.6
Drainage					
	water	300	40	10 min	
Drainage					
	water	200	40		
	Sellasol™ AG	8		45 min	
Addition	Luganil™ Beige L	6%		45 min	
Addition	water	100	60		
Addition	Lipoderm™ Licher SLW	11		45 min	
Addition	formic acid (96%) diluted 1:10	2.35		45 min	pH 3.6
Drainage					
Washing	water	300	50	10 min	
Drainage					
2'nd Dyeing	water	400	50		
	Luganil™ Beige L	1.3			
	Luganil™ Brown GOL	0.2		20 min	
Addition	formic acid (96%) diluted 1:10	0.94		30 min	pH 3.2

Addition	Lipoderm™ Licher SLW	1.0		15 min	
Drainage					
Washing	water	300	20	10 min	
Drainage					

Take out and dry. The product is referred to as crust leather.

Table 1

Grease Content and Acid Values after Soxtech™ Extraction

Treatment	Beaker	g Grease Extracted	%	Acid Value	Colour of Grease
Lipase absent	1	0.341		5.35	
	2	0.350		6.41	
	3	0.336		5.84	
	mean	0.342	17	5.87	bright yellow
Lipase present	4	0.138		111.59	
	5	0.137		109.54	
	6	0.140		111.00	
	mean	0.138	6.9	110.71	greenish black

CLAIMS

1. A process for enzymatic degreasing of skins and hides, which process comprises treatment of the skin or hide with a lipolytic enzyme at a pH below 5.

2. The process according to claim 1, which process is applied to ovine skins, to porcine skins, to bovine hides, or to caprine skins.

3. The process according to either of claims 1-2, wherein the lipolytic treatment takes place in an aqueous medium in the presence of a surfactant.

4. The process according to claim 3, in which the surfactant is an anionic, a non-ionic, or an amphoteric type surfactant, or a mixture thereof.

5. The process according to any of claims 1-4, which takes place during one or more of the subsequent steps of bating, pickling and tanning.

6. The process according to any of claims 1-4, which takes place as a separate step after pickling, or as a separate step after tanning.

7. The process according to any of claims 1-6, which process is carried out at a pH in the range of from pH 2 to pH 4.5.

8. The process according to claim 7, which process is carried out at a pH in the range of from pH 3 to pH 4.

9. The process according to any of claims 1-8, which process is carried out at a temperature in the range of from 20 to 65°C.

10. The process according to claim 9, which process is carried out at a temperature in the range of from 25 to 55°C.

11. The process according to any of claims 1-10, wherein the reaction time is in the range of ½-4 hours.

12. The process according to claim 11, wherein the reaction time is in the range of ½-2 hours.

13. The process according to any of claims 1-12, in which the lipolytic enzyme is a lipase derived from *Candida*, *Humicola*, *Hyphozyma*, *Pseudomonas*, or *Thermomyces*.

14. The process according to claim 13, in which the lipolytic enzyme is a lipase derived from *Candida antarctica*, *Candida cylindracea*, *Pseudomonas cepacia*, or *Thermomyces lanuginosus*.

15. The process according to any of claims 1-14, in which process after the lipolytic treatment the skins and hides are separated from the aqueous medium and subjected to one or more subsequent washes with water, optionally in the presence of a surfactant.

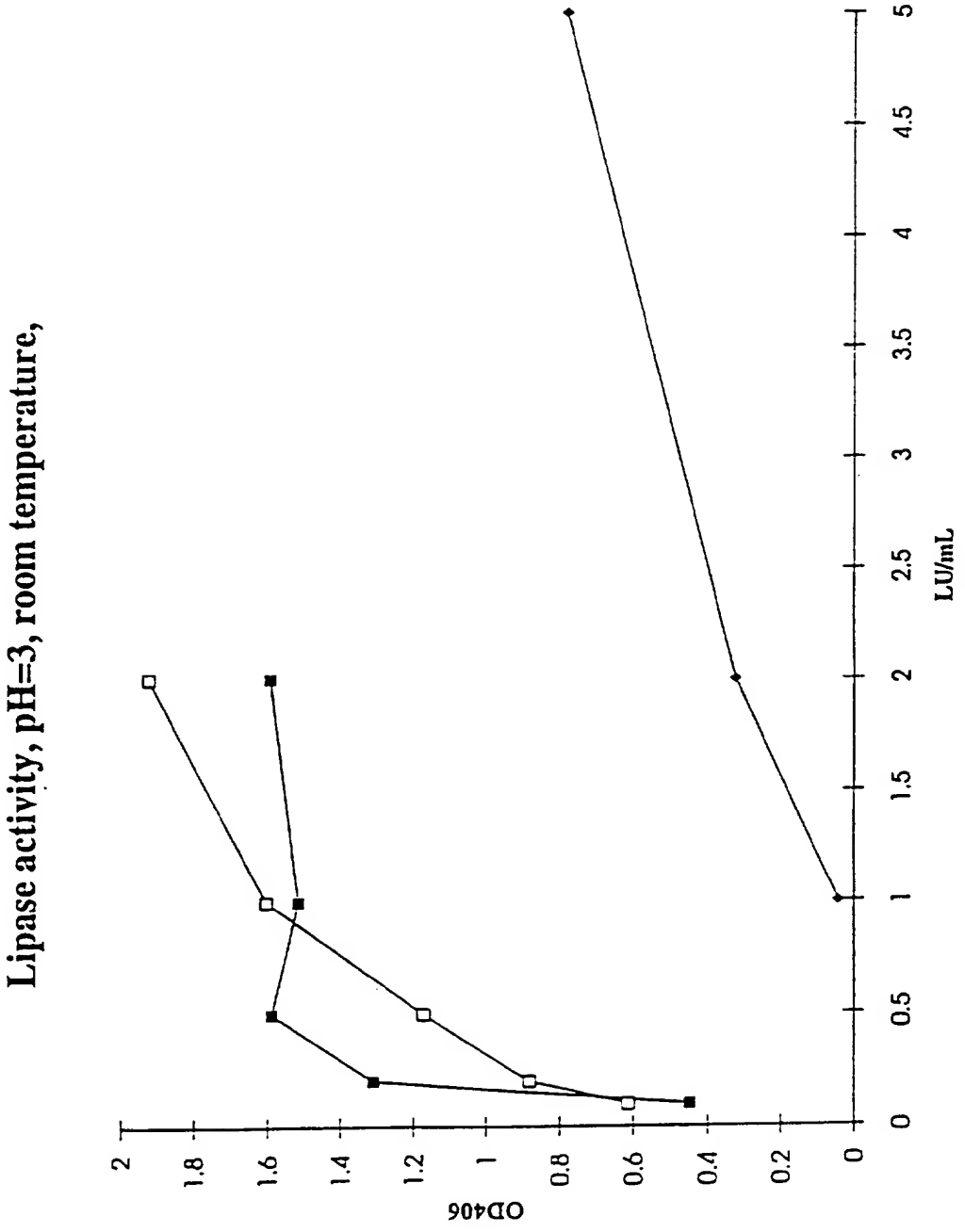
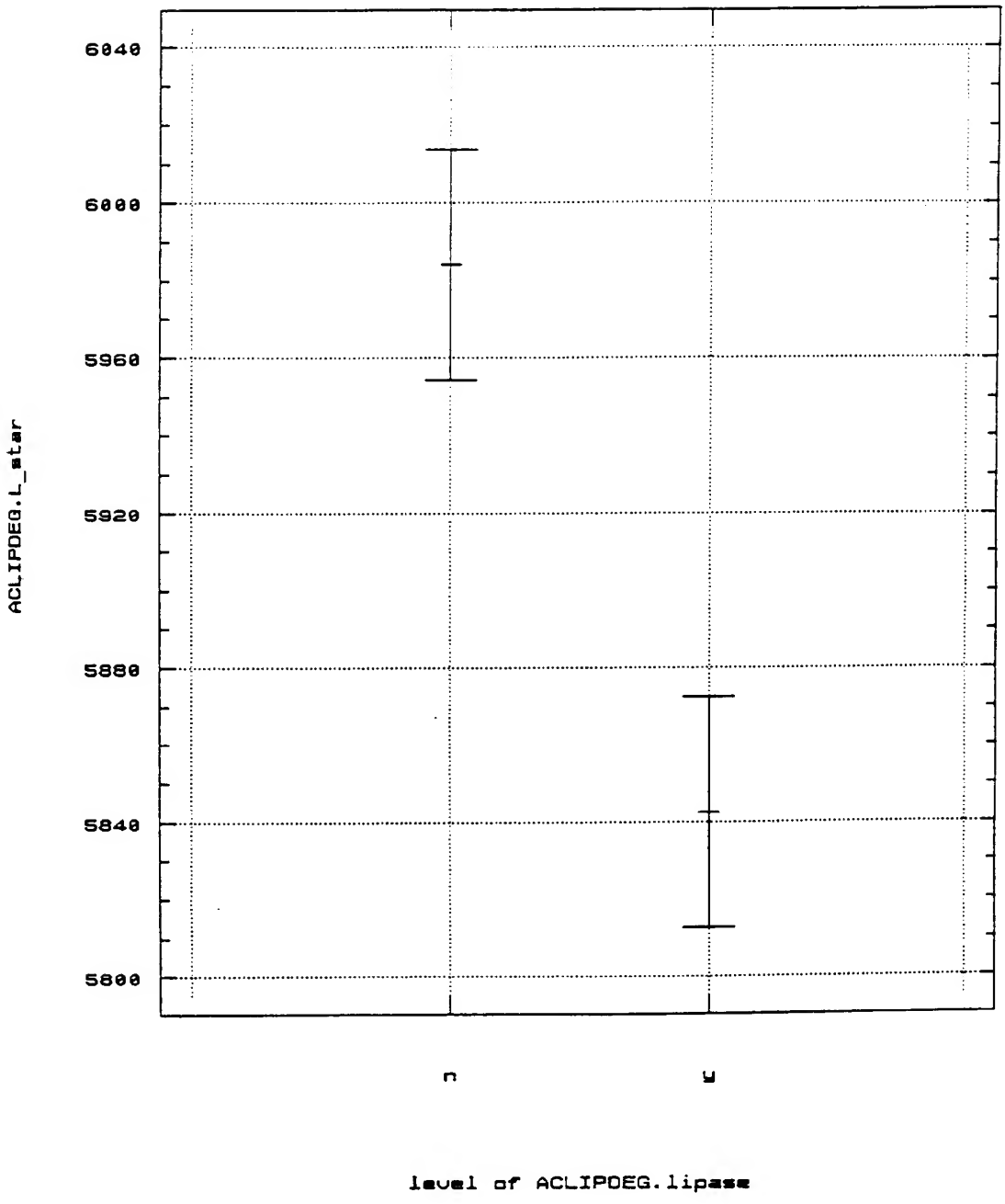

Lipase activity, pH=3, room temperature,
OD406
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
LU/mL

Fig. 1


6040
6000
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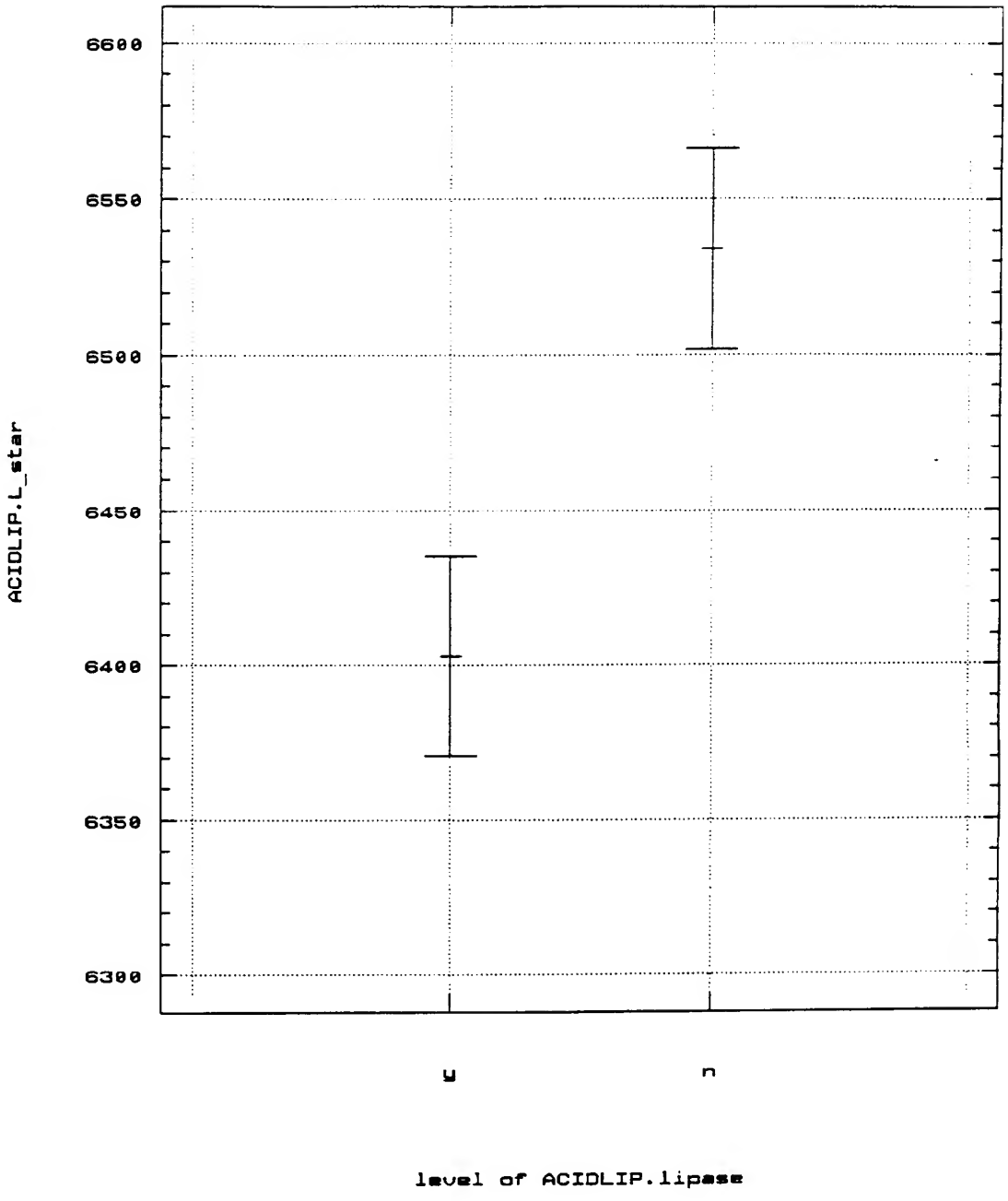
5880
5840
5800
ACLIPDEG.L_star
n
y
level of ACLIPDEG.lipase

Fig. 2


6600
6550
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6450
6400
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6300
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y
n
level of ACIDLIP.lipase

Fig. 3

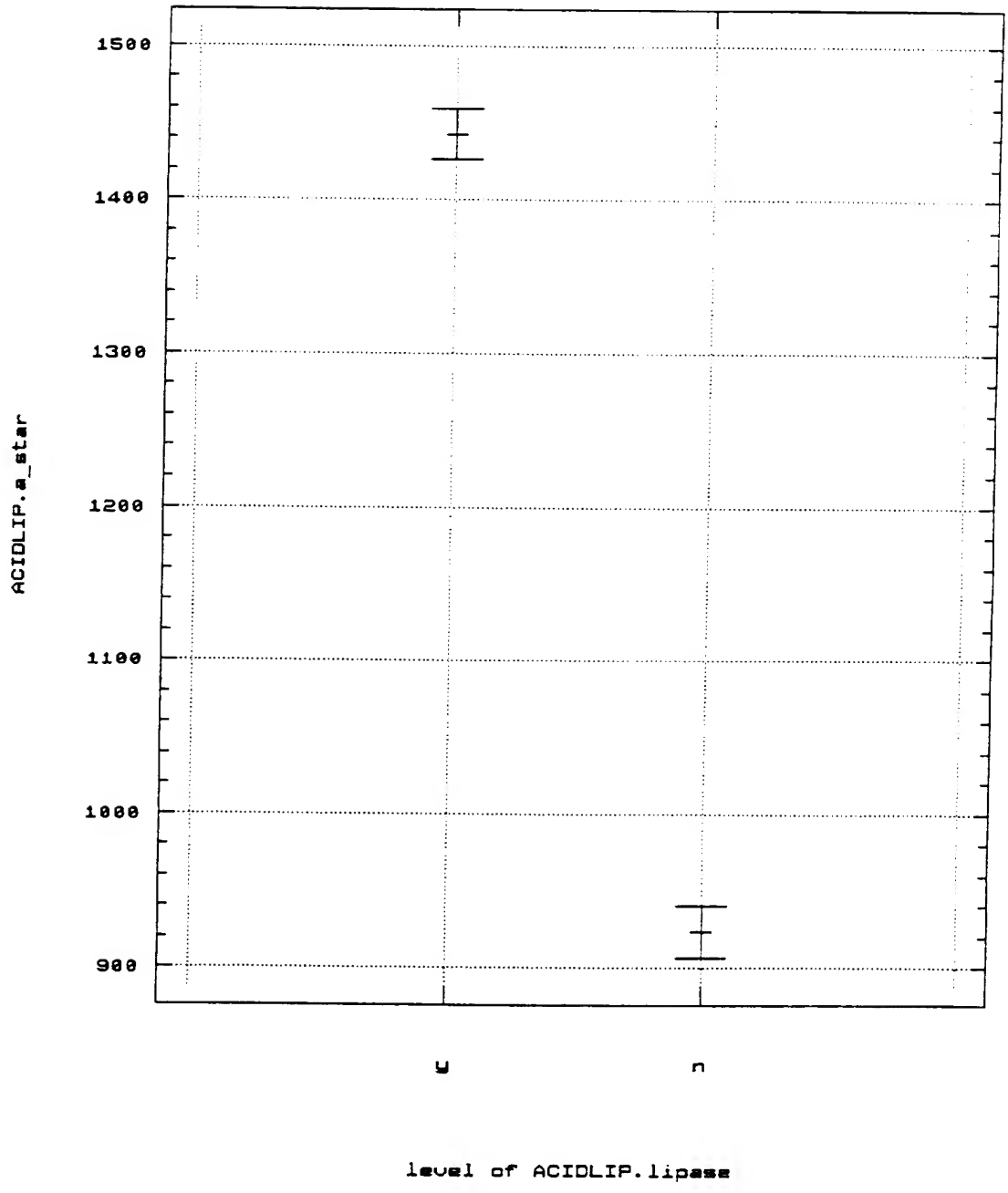
1500
1400
1300
1200
1100
1000
900
ACIDLIP.a_star
y
n
level of ACIDLIP.lipase

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 95/00464

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C14C 1/08, C12S 7/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C14C, C12N, C12S

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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CA SEARCH, WPI, CLAIMS/US PATENTS, JAPIO, EPODOC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Chemical Abstracts, Volume 89, No 24, 11 December 1978 (11.12.78), (Columbus, Ohio, USA), Yeshodha, K. et al., "Studies on the degreasing of skins using a microbial lipase", page 80, THE ABSTRACT No 199097c, Leather Sci.(Madras) 1978, 25 (2), 77-86, (Eng.)	1-2,9-12
X	Chemical Abstracts, Volume 98, No 5, 31 January 1983 (31.01.83), (Columbus, Ohio, USA), Muthukumaran, N. et al., "Comparative studies on the degreasing of skins using acid lipase and solvent with reference to the quality of finished leathers", page 102, THE ABSTRACT No 36450j, Leather Sci. (Madras) 1982, 29 (9), 417-24, (Eng.)	1-2

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
6 March 1996	12 -03- 1996
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer INGA-KARIN PETERSSON Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 95/00464

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